

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

## Flexible (Kapton-based) Microsensor Arrays of High Stability for Cardiovascular Applications

Ernö Lindner, Vasile V. Cosofret, Stefan Ufer and Richard P. Buck\*

Department of Chemistry, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Robert P. Kusy

Department of Orthodontics and Biomedical Engineering, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

R. Bruce Ash and H. Troy Nagle

Department of Electrical and Computer Engineering, North Carolina State University, Raleigh, NC 27695, USA

The design, fabrication and performance characteristics of Kapton-based planar mini and semimicro potentiometric sensors with an Ag/AgCl or a quinhydrone-based redox internal reference electrode are described. The ion-selective membranes cast from conventional and various modified PVC matrices and containing different pH-sensitive ionophores are ranked on the basis of their performances in hostile environments. The adhesive bonding strength of the different PVC membranes to the polyimide-coated Kapton substrate was quantitatively evaluated as a function of fabrication procedure and sample solution contact. The long-term stability of the electrodes was characterized by the alterations of the analytical parameters of the sensors over a period of time, as well as by determining the resistance changes of their sensing membranes.

Chemical sensors are important tools in cardiac electrophysiology.<sup>1</sup> Ischaemic events have ionic markers, specifically hydrogen and potassium ions, with characteristic and reproducible time courses of change after brief periods of ischaemia. Consequently, a number of investigators have applied ion-selective electrodes to measure changes in myocardial extracellular  $K^+$  and  $H^+$  activities in the intact beating heart.<sup>2</sup> Chemical consequences of fibrillation and defibrillation also require *in vivo* monitoring of ionic species, especially  $H^+$ ,  $K^+$ ,  $Na^+$  and  $Ca^{2+}$ . A major challenge is the selection of biocompatible materials for the development of manufacturing technology and sensor products for acute *in vivo* monitoring and for chronic applications in cardiology, in vascular diagnostics and intervention.<sup>3</sup> ~~The sensor array for monitoring ionic concentrations in the beating heart must be flexible~~ and have a known spatial resolution. In addition, there are a number of general requirements for *in vivo* applications (i.e. theoretical response, excellent stability and reproducibility, fast response etc.). The micropipette-type electrodes with tip diameters down to o.d. = 0.05  $\mu m$  are very delicate tools with extremely high resistance.<sup>4</sup> Pipette-type sensors with larger outer diameter (o.d.  $\geq 10 \mu m$ ) are less fragile but may cause serious cell damage as a result of their rigidity. Rigidity is one of the major disadvantages of the silicon-based sensors in this particular application. However, they have a number of other drawbacks (e.g. drifting potentials, fouling encapsulation, lack of small size reference electrode integrated on the same silicon substrate) which limit the exploitation of the theoretical advantages of semiconductor-based ISFETs. The drifting offset voltage of coated wire electrodes makes a high-precision measurement illusory when there is no possibility of frequent calibration (the general case for *in vivo* applications). Furthermore, because fabrication is very complex, the exact placement of a large number of sensors (which are needed for spatial resolution and experimental protocol) is very difficult.<sup>2</sup>

Since all the available mini and micro ion-selective electrode designs (micropipette type, coated wire type and

ISFETs) had several drawbacks with respect to cardiac application, efforts were made to design a flexible sensor with several sensing spots of well defined geometrical arrangement.<sup>5</sup> Our goal was to adapt existing semiconductor and microelectronics technologies to produce closely spaced sensor arrays on a flexible substrate that moves with the muscle fibres within the wall of a beating heart. ~~For this purpose 0.125 mm thick sheets of polyimide (PI), which were made by DuPont (Kapton), with outstanding insulation properties were selected.~~ A further advantage of Kapton-based wafers is offered by an easy encapsulation technique using polyimide as an insulating layer since excellent adhesion is attainable between the Kapton wafer and the polyimide coating.<sup>6,7</sup> The importance of encapsulation cannot be exaggerated since the majority of device failures may be traced directly to the encapsulant used.<sup>8</sup> Naturally, the adhesion of the sensitized membrane layers to the polyimide coating is also of great importance. To fabricate sensors of extended lifetime, first the potentiometric behaviour of several modified PVC matrices with improved adhesion properties was tested.<sup>9,10</sup> The adhesion strength between the PI-coated Kapton substrate and the sensing membrane was quantitatively evaluated in this work. Beside the effect of the membrane matrix, the influences of a short transient heat treatment in the processing and of aqueous electrolyte contact on the adhesion strength were also studied.

To improve the potentiometric response with respect to short- and long-term stability of the new planar sensor, the classical Ag/AgCl internal reference system was compared with a quinhydrone-based redox reference element. The latter had considerably smaller resistance, which may be crucial in micro electrode fabrication. In our work poly-HEMA (poly 2-hydroxyethyl methacrylate) saturated with an appropriate internal filling solution was generally used as a 'thermodynamically well defined' internal contact to the membrane.<sup>11</sup> However, planar electrodes without an electrolyte gel and with a cellulose acetate/ $TiO_2$ -based sponge<sup>2</sup> were also prepared, and the potential stability data were compared.

## Experimental

### Chemicals

For all experiments, deionized water doubly distilled in Pyrex glass and chemicals of puriss or pa grade were used.

### Fabrication of the Kapton Wafers with Sensor Regions from 100 to 2000 $\mu\text{m}$

The procedure can be broken up into three sections: (i) wafer fabrication, (ii) wire connections, and (iii) electrode site preparation.

#### Wafer Fabrication

Kapton squares or wafers 7.6 cm on each side (corresponding to our photomask area) were cut out and thoroughly cleaned with a series of solvent rinses (methanol/acetone/methanol/*N*-methyl-2-pyrrolidone(NMP)/distilled water) followed by a dry etch in an oxygen-argon plasma. 20 nm of chromium (an adhesion layer between the Kapton and gold) followed by 200 nm of gold were sputtered onto one side of the wafers without braking the vacuum. Using standard lithography process, positive photoresist was patterned onto the metal to define the electrode arrays including their bonding pads and interconnects. The patterned gold and chromium films were etched in successive chemical etches in commercial etch solutions. After stripping off the photoresist using NMP and dehydrating the wafers on 125°C hotplate for 30 min, a 2  $\mu\text{m}$  layer of Du Pont PI 2570-D polyimide was spun onto the wafers. Openings for the electrode sites and bonding pads were patterned into the uncured polyimide using the same photolithography process and etching the polyimide in one part Shipley 351 developer and five parts deionized water. After removing the photoresist with acetone and methanol rinses, the polyimide was cured for 6 h on a hotplate at 300°C.

#### Wire Connections

The wire connections were made by using silver paint and were strengthened by a non-conducting epoxy over the paint bonds. Following the complete cure of the epoxy, a layer of silicone rubber was applied over the entire bonding area to insulate all wire exposed to water contact as well as to add additional mechanical stability.

#### Electrode Site Preparation

After cleaning the electrodes (methanol/acetone/methanol/distilled water) the procedure was different for the Ag/AgCl and the quinhydrone-based internal reference system, but always the procedure suggested in ref. 12 was followed. For silver plating a 10 g l<sup>-1</sup> KAg(CN)<sub>2</sub> solution (Pfaltz and Bauer Inc., S03370 and 0.4 mA cm<sup>-2</sup> current density was applied for 3 h. The silver spots were chloridized in 0.1 mol l<sup>-1</sup> HCl solution at the same current density for 30 min. To prepare a quinhydrone based reference electrode the gold electrode spots were platinized in a commercial platinizing solution (Malinkrodt H314) containing 1.5 g PtCl<sub>6</sub> · 6H<sub>2</sub>O and 0.125 g lead acetate in 50 ml solution. A current density of 100 mA cm<sup>-2</sup> was applied for 1 min, which resulted in a thick, porous Pt black layer.

### Membranes and Membrane Solutions

The solvent polymeric membrane cocktail composition was 1 wt.% ionophore, 64–66 wt.% plasticizer and 33 wt.% PVC and 70 mol.% (compared to the ionophore) potassium tetrakis(*p*-chlorophenyl)borate (KTpClPB) as lipophilic salt additive dissolved in tetrahydrofuran or cyclohexanone. However, in some cases membranes without ionophore (mainly aminated PVC membranes) were also cast. All iono-

phores and plasticizers were products of Fluka AG (Buchs, Switzerland): tridodecylamine (TDDA) (Fluka 95292); ETH 5294 (Fluka 27086); 2-nitrophenyl octylether (o-NPOE) (Fluka 73741); bis(2-ethylhexyl) sebacate (DOS) (Fluka 84818). As polymeric membrane materials, several commercially available products were tested: poly(vinyl chloride) (PVC, high molecular weight, HMW), Fluka 81392, carboxylated PVC (PVC-CO<sub>2</sub>H, with 1.8% CO<sub>2</sub>H groups), Aldrich 18955 and hydroxylated PVC (PVC-OH), a copolymer of 91% vinyl chloride, 3% vinyl acetate and 6% vinyl alcohol, Fluka 27827. The last could not be used alone for membrane casting (as a consequence of the bad mechanical properties of the membrane) but only as a 1:1 mixture with HMW. The 1,4-diaminobutane (1,4-DAB) and piperazine (PIP) modified PVC samples were prepared in our laboratories with the reaction scheme suggested by Ma and Meyerhoff.<sup>13</sup>

### Fabrication of Planar Ion-selective Electrodes on Kapton Wafers

First, the Pt coated spots were impregnated with a methanol solution containing 100 mg ml<sup>-1</sup> quinhydrone. Next, both the Ag/AgCl and Pt electrodes were saturated with 4–400 nl freshly prepared HEMA solution (2-hydroxyethyl methacrylate solution with 4.0% 2,2-dimethoxy-2-phenylacetophenone) depending on the size of the electrode (the opening in the PI coating, 0.25–2.0 mm). The HEMA solution was then illuminated with an Mineralight UVSL 25 short and long wave lamp (Ultra-Violet Products Inc.) at 365 nm for 2h. Following the polymerization of the HEMA the electrodes were soaked in a pH 7.00 Tris buffer solution containing 0.14 mol l<sup>-1</sup> NaCl and saturated with quinhydrone in the case of the redox reference electrode. Following a brief washing with distilled water and gentle wiping of the surface, the membrane cocktail was applied above the active area using a General Valve Picosprizer II or a World Precision Instrument Nanoliter Injector (A203XVY). After a complete evaporation of the solvent used to dissolve the membrane components (THF or cyclohexanone) the electrodes were presoaked in a conditioning solution (pH 7.00 Tris) for 12–24 h before use.

### Buffer Solutions

To determine the pH sensitivity, short- and long-term stability, reproducibility *etc.* of the planar electrodes the following buffer solutions were used: (I) citrate-borate buffer with 60 mmol l<sup>-1</sup> Li<sup>+</sup> ion background (60 mmol l<sup>-1</sup> LiOH, 6.6 mmol l<sup>-1</sup> citric acid and 11.4 mmol l<sup>-1</sup> boric acid); (II) a Tris buffer with 140.0 mmol l<sup>-1</sup> Na<sup>+</sup> ion background [10.0 mmol l<sup>-1</sup> NaOH, 130.0 mmol l<sup>-1</sup> NaCl and 10 mmol l<sup>-1</sup> Tris(hydroxymethyl)aminomethane]; (III) a Tris buffer with 200.0 mmol l<sup>-1</sup> K<sup>+</sup> ion background [190.0 mmol l<sup>-1</sup> KCl, 10.0 mmol l<sup>-1</sup> KOH and 10 mmol l<sup>-1</sup> Tris(hydroxymethyl)aminomethane]; (IV) a Britton Robinson buffer with or without 140 mmol l<sup>-1</sup> NaCl (see Table 10.47 in ref. 14). The pH values of the solutions were adjusted by means of a hydrogen-selective glass electrode (Orion model 91-57) by addition of hydrochloric acid or sodium hydroxide to the stock buffer solutions.

### EMF Measurements

Cell voltages were measured at room temperature in an air-conditioned laboratory at 22.5 ± 0.5°C with an Orion Expandable Ion Analyzer (Model EA 920) connected to an Orion model 607 manual electrode switch. As a reference electrode an Orion model 90-02 Ag/AgCl double-junction reference electrode was used throughout. The measured potential values were recorded with an ABB Goertz 420 strip

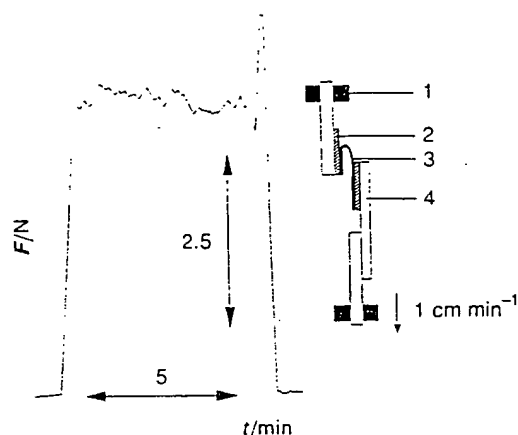


Fig. 1 Schematic representation of the experimental arrangement to evaluate the 180° peel-off test (right) and a typical force-time graph (left) recorded with a PVC-OH membrane-coated Kapton substrate after a short heat treatment. 1, Pneumatic grips; 2, Kapton foil; 3, PVC membrane; 4, sample holder

chart recorder. The selectivity coefficients  $K_{H,M}^{pot}$  were determined by the fixed interference method (FIM)<sup>15</sup> using pH buffer solutions I–III and the data were compared to those gained with conventional macroelectrodes fabricated with the same membrane.

#### Evaluation of Membrane Adhesion: Peel-off Tests

For testing the adhesion of different modified PVCs to PI-coated Kapton surfaces, first square-shaped Kapton samples (7.6 cm × 7.6 cm) were cut and scratched on their back side as deep as 4/5 of the sample thickness, dividing the sample in halves. The Kapton sample surfaces were cleaned (methanol/acetone/methanol/dehydration bake at 120°C) and several layers of PVC or modified PVC (e.g. PVC-CO<sub>2</sub>H, PVC-NH<sub>2</sub>, PVC-OH) were spun on the sample at a spinning rate of 1500 rpm. The composition of the solution used for PVC spinning was 1.2 g PVC, 0.4 g *o*-NPOE in 20 ml cyclohexanone. The approximate PVC membrane thickness was calculated on the basis of the weight increase of the samples.<sup>16</sup> Before the peel-off tests, the PVC coated Kapton samples were bent 180° along the scratched line. As a result the Kapton sample split into two pieces but was held together by the flexible (plasticized) PVC coating. Next, 1.25 cm wide strips of the samples were fixed to sample holders and were mounted into an instrument designed to measure the tensile strength of samples (Instron Testing Machine, Canton, MA) in order to determine the force needed to peel off the membranes at a crosshead extension rate of 1 cm min<sup>-1</sup> (Fig. 1). Since our preliminary results showed that some heat treatment of PVC coated Kapton samples contributes to considerable increase of the adhesive bonding strength, selected samples were heated up to 120°C for 20 min, and the peel-off tests were carried out parallel with the

non-heated samples for comparison. Since alterations in the adhesive strength were expected as an effect of water absorption of the membranes in use,<sup>17</sup> several of the PVC-coated samples were soaked in pH 7.00 Tris buffer for 1 week before the peel-off tests were performed (Table 1).

#### Determination of the Internal Resistance of the Cells

The internal resistances of the cells were determined by the voltage divider method using known shunts.<sup>18</sup>

### Results and Discussion

#### Ranges, Slopes and Selectivities of the Membranes

In our work the hydrogen ion-selective membrane was selected as a model based on its primary importance in cardiology and on the accumulated knowledge and experience concerning its biological applications. By this choice our results on planar electrodes could be compared both to macroelectrodes fabricated from the same membrane and to the pH glass electrode, accepted throughout the world as 'standard'. A comparison to the pH glass electrode was especially important in studies concerning the electrode behaviour in a hostile environment.

Before constructing the Kapton based planar electrodes, the H<sup>+</sup> ion-selective membranes were tested and compared thoroughly.<sup>9,19,20</sup> Among the analytically important properties, several (e.g. linear range, slope, selectivity) are hardly influenced by the electrode construction or design. As expected, the above parameters were found to be the same with the conventional macro and the new planar electrodes. The differences found were due to the differences of the ionophore nature (TDDA, ETH-5294, ETH 5350, ETH 2439), plasticizer (*o*-NPOE, DOS), and membrane matrix (PVC-HMW, PVC-CO<sub>2</sub>H, PVC-OH, PVC-NH<sub>2</sub>) as well as to the composition of the buffer solution selected for the experiment.<sup>9,20</sup> Based on the results in aqueous electrolyte solutions that mimic blood serum, all combinations (ionophore/matrix/plasticizer) seemed to be appropriate for high-precision pH measurements in the physiological pH range. The slopes were always close to the theoretical value [ $S = (58.0 \pm 1.5) \text{ mV/pH}$ ] and the selectivity coefficients were adequate for biological applications considering the normal ranges for interferences.<sup>21</sup>

#### Electrode Behaviour in Hostile Environment

After testing the membranes in synthetic aqueous blood serum-like electrolytes, but before testing them on the planar electrode design, the effects of the hostile environment on the membranes of different compositions were studied in conventional macroelectrodes. The model solutions were as follows: 7% bovine serum albumin, 10% glucose, 10% glycine, 10% sucrose and 80% blood serum solution centrifuged from pig blood. The pH value of all these solutions was kept constant at pH 7.00 using a 0.1 mol l<sup>-1</sup> BES buffer. To avoid pH changes in the blood serum samples (controlled by the hydrocarbonate/CO<sub>2</sub> equilibrium) 20 v/v% of BES buffer was added to 80% blood serum sample. The potential stability (drift) and the residual standard deviation of data around the line were determined with the selected synthetic solutions. Generally 16–20 data points were collected within the time period of the stability measurements (2 h). The slope and  $E^0$  values of the electrodes were checked before and after immersing them into the corresponding test solution.

On the basis of the results it can be stated that no serious interference from the above model solutions had to be considered. The drift values in many model solutions ranged

Table 1 Peel-off forces (in N) of different PVC membranes from polyimide-coated Kapton substrate<sup>a</sup>

sample treatment	membrane matrix			
	PVC-CO <sub>2</sub> H	PVC-NH <sub>2</sub>	PVC-OH	PVC-HMW
unheated	1.0 ± 0.05	1.2 ± 0.05	0.8 ± 0.05	<0.1
heated	9.5 ± 0.5	9.5 ± 0.5	5.0 ± 0.2	<0.1
heated and soaked	3.2 ± 0.2	2.5 ± 0.1	1.5 ± 1	<0.1

<sup>a</sup> The data are the average values of at least three parallel measurements.

from 0.1 to 2.5 mV h<sup>-1</sup> with a standard deviation of 0.1–0.8 mV. The  $E^0$  values (before and after contacting the electrode with the model solution for at least 2 h) were changed from 0.1 to 2.6 mV while the alterations in the slope values ranged from  $\pm 0.1$  to  $\pm 2.8$  mV/pH. The original slopes were restored after soaking the electrodes for a short time in an aqueous buffer solution. In spite of these encouraging results, we intended to rank order the electrodes from 1 to 10 considering the individual properties studied in all solutions involved in the experiment. First place was given to the electrode with the smallest drift or with the smallest standard deviation *etc.* When the grades were summed, the electrodes were ranked as follows: PVC-NH<sub>2</sub>/TDDA  $\gg$  PVC-CO<sub>2</sub>H/TDDA  $\approx$  PVC-OH/ETH-5294  $\approx$  glass > PVC-NH<sub>2</sub>/ETH-5294 > PVC-CO<sub>2</sub>H/ETH-5294 > PVC-OH/TDDA > PVC-HMW/TDDA > PVC-HMW/ETH-5294  $\gg$  PVC-NH<sub>2</sub> (without ionophore).

We are aware of the limitations of this evaluation (the ranking enlarges small differences in the properties considered, but conceals some important ones; and we cannot make conclusions among the important and less substantial properties *etc.*), but some trends are worthwhile noting. The aforementioned ranking of the membranes can be grouped into three categories (divided by two 'larger' signs:  $\gg$ ). PVC-NH<sub>2</sub>/TDDA rose above the others in almost every respect (in all model solutions and all parameters considered), while the piperazine-based aminated PVC without additional ionophore fell short of our expectations.<sup>19</sup> The combination of the pH-sensitive aminated PVC with a pH-sensitive ionophore seems to be a beneficial choice (both PVC-NH<sub>2</sub> based electrodes compounded with TDDA or ETH 5294 are on the top of the list). The differences between the membranes in the second group are insignificant. The effect of the ionophore incorporated into the PVC matrix ranged from unimportant (HMW) to relatively large (PVC-OH) with intermediate examples (PVC-NH<sub>2</sub> and PVC-CO<sub>2</sub>H). It is interesting that conventional HMW membranes are at the end of the list. In

general the modified matrices in combination with TDDA seem to be beneficial compared to HMW PVC. TDDA proved to be better compared to ETH 5294 in all matrices (except in PVC-OH) in 'hostile environment'.

### Planar Electrode Design

The layer structure of the Kapton-based wafers is shown in Fig. 2, while some electrode designs are summarized in Fig. 3. By decreasing the overall dimensions of the sensor and, parallel to this the size of its active area, the contact surface between the wafer and the sensitive membrane is decreased dramatically. In other words the adhesion between the wafer and sensitive membrane often becomes the limiting factor of the lifetime of the sensors.

Among the different possibilities for increasing the adhesion between the wafer and the membrane, the use of aminated PVCs seems to be especially attractive. The surface of partially cured polyimide contains some free reactive groups from polyamic acid which offer a possible covalent coupling. This coupling was exploited by using organosilane couplers to increase the adhesion of PI to silicon dioxide surfaces.<sup>6</sup> However, a similar coupling is possible by using modified PVC samples containing free NH<sub>2</sub> groups (Scheme 1). Unfortunately, aminated PVCs cannot always be used as the membrane matrix. Their pH sensitivity is an advantage in ionophore-free pH sensors,<sup>19</sup> but gives rise to serious H<sup>+</sup> ion interference when used as membrane matrix material in fabricating other cation-selective membranes (K<sup>+</sup>, Ca<sup>2+</sup> *etc.*). To find the best selection among the possible membrane materials, besides their analytical<sup>9</sup> and mechanical<sup>22</sup> characteristics, their adhesive properties were tested in detail as well. As is seen from Table 1, the adhesion is considerably better for the modified PVC samples (PVC-NH<sub>2</sub>  $\approx$  PVC-CO<sub>2</sub>H > PVC-OH  $\gg$  HMW). Another important observation is that the adhesive strength increased by about one order of magnitude as a result of the short heat treatment. Unfortunately,

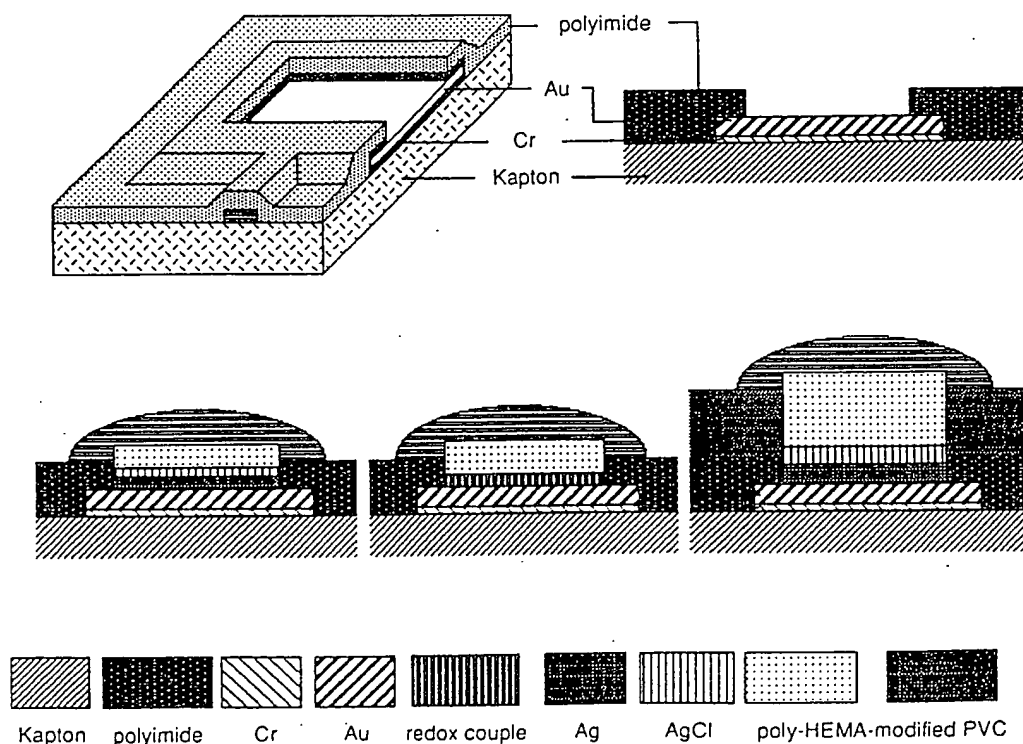
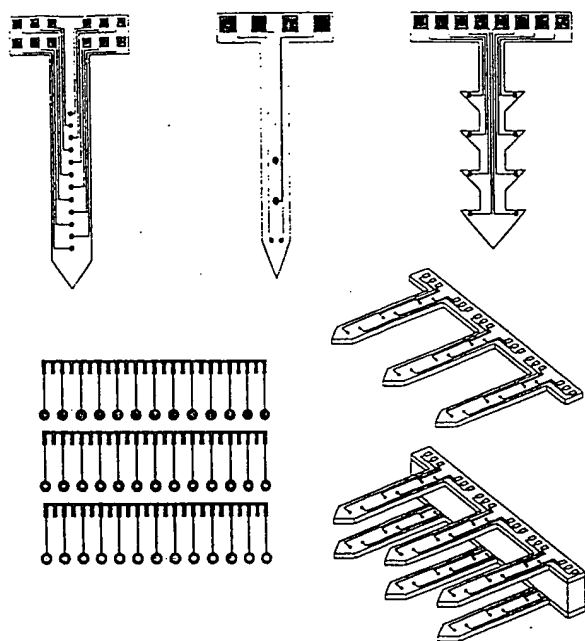


Fig. 2 Layer structures of various Kapton-based wafers and planar electrodes



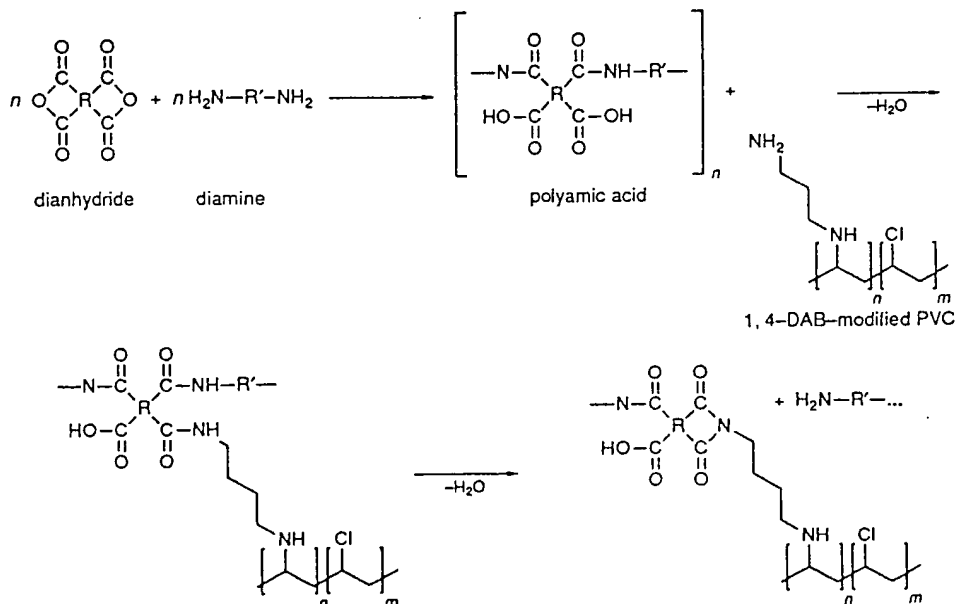
the gap in the adhesive strength between the heated and unheated samples decreased after soaking the samples in aqueous electrolytes.

Fortunately, no weight loss could be detected as a result of the short heating of the membranes (no loss of plasticizer), and the analytical properties of the electrodes made from heated membranes were similar to the unheated ones (no ionophore degradation). The only disadvantage of the heat treatment is that the water-equilibrated, swollen poly-HEMA below the membrane is dried out. Consequently, a longer hydration procedure (conditioning period) is needed with such electrodes (see Fig. 5, later). This drawback could be reduced by forming a doughnut-like crater from a slightly plasticized modified PVC (without ionophore) around the active spot of the wafer and applying the heat treatment only to this PVC 'spacer'. In this way the time of the heat treatment can be extended, which is beneficial to adhesion,

without exposing the active membrane to relatively high temperatures. Next, the crater is filled with HEMA. The HEMA can be polymerized and wetted in the crater. Finally, the active membrane is cast above the crater. Excellent adhesion is expected between the doughnut-like PVC ring and the PVC membrane, while good adhesion was assured between the wafer and the PI-coated Kapton by the appropriate PVC selection and the heat treatment. A further advantage of the PVC ring around the active surface is that it allows the amount of HEMA placed below the membrane to increase. This appears important with respect to the electrode stability.

### High-stability Internal Reference Electrodes

The miniaturization of chemical sensors has led to the development of potentiometric sensors with solid internal contact (coated wire sensors, ISFETs *etc.*).<sup>23</sup> Such electrodes often exhibit unacceptable drift and therefore were criticized both from practical and theoretical standpoints. The most encouraging approaches involve the interposition of a thin layer of aqueous gel that contains the primary ion at a constant concentration.<sup>2,24-26</sup> The gel (or only the internal filling solution) can be incorporated into porous solid materials for better mechanical properties and to slow down the diffusion processes, since neutral species tend to diffuse through the membrane and change the ionic activities within the gel.<sup>2,26</sup> To keep the ionic activities constant, the internal solutions are often saturated with an excess of solid material.<sup>24</sup> By constructing planar pH sensors the internal gel can be hydrated (wetted) by conditioning it in a buffer of high capacity. Using a redox couple-based reference system the pH should be controlled anyhow. In our work we compared the properties of a quinone/hydroquinone (quinhydrone)-based internal reference system to the more conventional Ag/AgCl system but no significant difference was found (Fig. 4). To hydrate the poly-HEMA, a pH 2.4 Britton-Robinson buffer or pH 7.00 Tris buffer was used but without any change in long-term stability. The pH 2.4 internal filling solution was selected, since some non-ideality of the quinhydrone electrode response was expected at higher pH values.<sup>12</sup> The pH 7.00 Tris buffer with 0.14 mol l<sup>-1</sup> NaCl aimed to approach the composition of blood serum. The resistance of a quinhydrone internal reference electrode-based planar ion-selective electrode was approximately one-half of that measured with the same



### Scheme 1

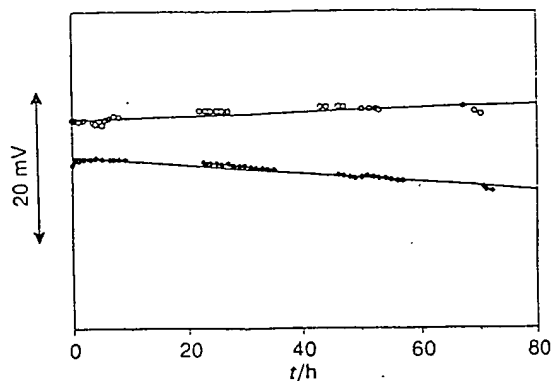


Fig. 4 Long-term stability of Kapton-based planar electrodes with Ag/AgCl ( $\blacklozenge$ ,  $-0.05 \text{ mV h}^{-1}$ ) or a redox couple-based (quinhydrone) internal reference electrode ( $\circ$ ,  $0.03 \text{ mV h}^{-1}$ ). The sensing membrane was fabricated from PVC-NH<sub>2</sub> as matrix and TDDA as ionophore

sensor but based on an Ag/AgCl internal reference electrode. The absolute resistance values were largely dependent on the conditions of sensor fabrication (e.g. size of the sensor, polymerization grade of poly-HEMA, ion-selective membrane thickness etc). The advantages of a redox internal reference electrode can be seen by the benefits of the Ross pH electrodes.<sup>27</sup>

#### Analytical Performance of Kapton-based Planar Electrodes in Comparison to Macroelectrodes with Massive Internal Solution

##### Short-term Stability, Reproducibility and Response Time

In contrast to the electrode characteristics discussed above there are several 'kinetically' controlled properties which were found to be different for planar and conventional macroelectrodes. These differences are assumed to be a consequence of the transport of neutral species such as H<sub>2</sub>O through the membrane and connected with changes of the ionic activities in the internal solution. This is seen on the much longer time needed for hydration of planar electrodes compared to conventional macroelectrodes in order to achieve high potential stability (Fig. 5). Furthermore, the potential vs. time transients, following a one unit pH change (response time curves) are slightly different for the conventional macro- and the planar mini-/micro-electrodes. The fast transient signal is generally followed by a small drift-like

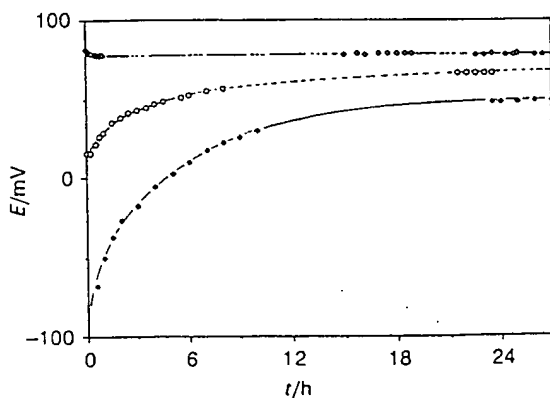


Fig. 5 Potential vs. time transients for conventional macro- and two different planar mini-electrodes during the hydration (wetting) period. The sensing membrane consisted of ETH 5294/PVC-NH<sub>2</sub>/o-NPOE. In the case of 'heated' electrodes both the sensing membrane and the hydrated poly-HEMA were subjected to transient heat treatment.  $\circ$ , Macro-electrode;  $\diamond$ , planar mini-electrode, unheated;  $\blacklozenge$ , planar mini-electrode, heated

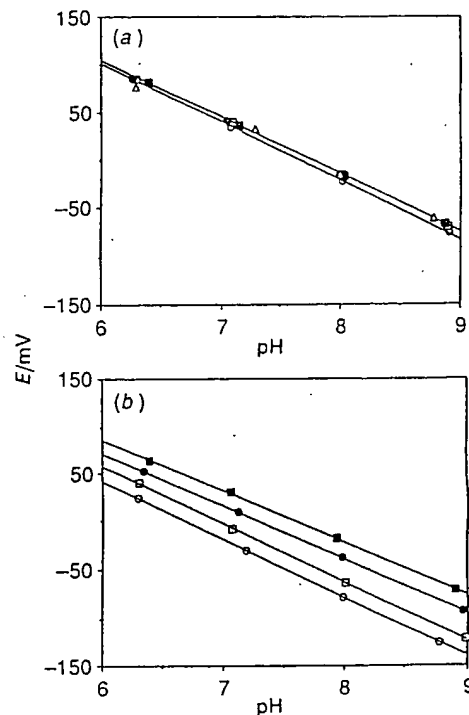


Fig. 6 Potentiometric response of Kapton-based planar pH electrodes ( $\phi = 0.5 \text{ mm}$ ) in extended physiological range. (a) TDDA/PVC-NH<sub>2</sub> membrane;  $\circ$ , day 1;  $\square$ , day 3;  $\bullet$ , day 9;  $\blacksquare$ , day 23;  $\triangle$ , day 37; (b) ETH 5294/PVC-NH<sub>2</sub> membrane;  $\circ$ , day 1;  $\square$ , day 6;  $\bullet$ , day 11;  $\blacksquare$ , day 26

potential change by the planar electrodes, but no such drift was found for the macroelectrode.<sup>28</sup> This difference in their transient behaviour is the reason for the better reproducibility of the macroelectrode. The smaller the activity step, the smaller is the difference expected in the transient response and in the reproducibility between the two electrode constructions. Fortunately, only slight pH changes are relevant for *in vivo* applications, i.e. the difference in the performance of the two constructions should be negligible.

##### Long-term Stability, Time-dependent Membrane Resistance, Lifetime

The correlation between the lipophilicity of the membrane components (ionophore, plasticizer, additives) and sensor lifetime was discussed in detail by Simon and co-workers.<sup>16,29</sup> In this regard we found that the membrane matrix has a

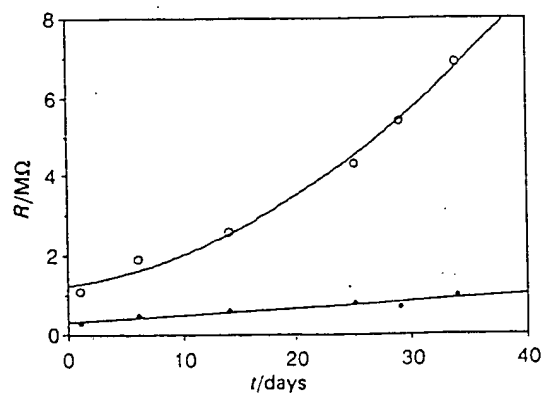


Fig. 7 Changes in the resistances of 2 mm size Kapton-based planar electrodes as a function of soaking time in a pH 7.00 Tris buffer solution:  $\circ$ , PVC-NH<sub>2</sub> (PIP)-ETH 5294;  $\blacklozenge$ , PVC-NH<sub>2</sub> (PIP)-TDDA

remarkable influence on the partition of the ionophore between the aqueous phase and the membrane phase.<sup>9</sup> The dissolved amount of Nile Blue A derivatives (phenoxazine-type pH-sensitive ionophores) was found to be considerably larger from aminated and carboxylated PVC in comparison to PVC-OH or PVC HMW. Furthermore, the dissolution rate of some pH-sensitive ionophores (phenoxazine type) is much larger in acidic than alkaline solutions.

The long-term stability of the planar electrodes was measured by calibration from time to time (Fig. 6). Between the tests the electrodes were kept in a pH 7.00 Tris buffer solution. No shift in the offset voltage ( $E^0$ ) was found within 37 days when TDDA was used as ionophore [Fig. 6(a)]. In contrast, a considerable  $E^0$  change was found with membranes containing ETH 5294 as the ionophore [Fig. 6(b)]. The alteration of the membrane resistances were similar to the changes of the  $E^0$  values. Relatively small increases in resistances were measured with the TDDA-based electrodes within this period of time, but considerable increases in resistances were determined with membranes containing ETH 5294 as ionophore (Fig. 7). The slopes of the calibration curves were practically constant in both cases.

### Conclusions

The analytical performance characteristics of Kapton-based planar mini and semimicro potentiometric sensors with an Ag/AgCl or a quinhydrone-based redox internal reference electrode are comparable to classical macroelectrodes with massive internal solution. The fabrication procedure described herein makes the large-scale manufacturing of planar ion-selective electrode arrays of high stability realizable in the near future.

Results on the analytical behaviour of various pH-sensitive membranes in hostile environments proved that modified PVCs may have distinct advantages over conventional high-molecular-weight PVC. However, some membrane matrix/ionophore combinations give better performance than others in the different environments we tested. The piperazine-based aminated PVC with TDDA as the ionophore is superior to other matrix/ionophore combinations in many respects (long-term stability, adhesion to the substrate, lifetime etc.).

This work was supported by the North Carolina Biotechnology Center and the Duke-North Carolina NSF Engineering Research Center for Emerging Cardiovascular Technologies. S.U. gratefully acknowledges financial support from the Department of Research and Technology of the Nordrhein-Westphalian Government in Germany.

### References

- 1 L. S. Gettes and W. E. Cascio, in *The Heart and Cardiovascular System*, ed. H. A. Fozzard, Raven Press, New York, 2nd edn., 1992, p. 2021.
- 2 T. A. Johnson, C. L. Engle, R. P. Kusy, S. B. Knisley, C. A. Graebner and L. S. Gettes, *Am. J. Physiol.*, 1990, **258**, H 1224.
- 3 R. P. Buck, V. V. Cosofret, T. M. Nahir, T. A. Johnson, R. P. Kusy, K. A. Reinbold, M. A. Simon, M. A. Neuman, R. B. Ash and H. T. Nagle, in *Biosensors and Chemical Sensors*, ACS Symp. Ser. No. 487, ed. P. G. Edelman and J. Wang, ACS, Washington DC, 1992, p. 237.
- 4 D. Ammann, *Ion-selective Microelectrodes, Principles, Design and Application*, Springer-Verlag, Berlin, 1986.
- 5 R. B. Ash, H. Z. Massoud, H. T. Nagle Jr. and J. J. Wortman, unpublished results.
- 6 D. S. Soane and Z. Martynenko, *Polymers in Microelectronics, Fundamentals and Applications*, Elsevier, Amsterdam, 1986.
- 7 W. M. Reichert and S. S. Saavedra, in *Materials Science and Technology*, ed. R. W. Cahn, P. Haasen and E. J. Kramer, Vol. 14, Medical and Dental Materials, Volume ed., D. F. Williams, VCH, Weinheim, 1992, p. 304.
- 8 W. H. Ko, in *Implantable Sensors for Closed-Loop Prosthetic Systems*, ed. W. H. Ko, Futura Publishing Company, Mount Kisco, NY, 1985, p. 259.
- 9 E. Lindner, V. V. Cosofret, R. P. Kusy, R. P. Buck, T. Rosatzin, U. Schaller, W. Simon, J. Jeney, K. Toth and E. Pungor, *Anal. Chem.*, submitted.
- 10 T. Stachwill and D. J. Harrison, *J. Electroanal. Chem.*, 1986, **202**, 75.
- 11 F. Oehme, *GIT Fachz. Lab.*, 1986(6), 595.
- 12 D. J. G. Ives and G. J. Janz, *Reference Electrodes, Theory and Practice*, Academic Press, New York, 1961.
- 13 S. C. Ma and M. E. Meyerhoff, *Mikrochim. Acta I*, 1990, 197.
- 14 D. D. Perrin and B. Dempsey, *Buffers for pH and Metal Ion Control*, Chapman and Hall, London, 1974.
- 15 P. L. Bailey, *Analysis with Ion-selective Electrodes*, Heyden, London, 2nd edn., 1980, ch. 3, p. 49.
- 16 U. Oesch and W. Simon, *Anal. Chem.*, 1980, **52**, 692.
- 17 D. J. Harrison, X. Li and S. Petrovic, IEEE Int. Conf. on Solid State Sensors and Actuators, Tech. Digest, p. 777, San Francisco, June, 1991.
- 18 D. Ammann, E. Pretsch, W. Simon, E. Lindner, A. Bezegh and E. Pungor, *Anal. Chim. Acta*, 1985, **171**, 119.
- 19 V. V. Cosofret, E. Lindner, R. P. Buck, R. P. Kusy and J. Q. Whitley, *J. Electroanal. Chem.*, 1992, in the press.
- 20 V. V. Cosofret, T. M. Nahir, E. Lindner and R. P. Buck, *J. Electroanal. Chem.*, 1992, **327**, 37.
- 21 U. Oesch, P. Anker, D. Ammann and W. Simon, in *Ion-selective Electrodes*, 4, ed. E. Pungor, Akadémiai Kiadó, Budapest, 1985, p. 81.
- 22 R. P. Kusy *et al.*, unpublished results.
- 23 J. Janata, *Principles of Chemical Sensors*, Plenum Press, New York, 1989.
- 24 F. Keplinger, R. Glatz, A. Jachimowitz, G. Urban, F. Kohl, F. Olcaytug and O. J. Prohaska, *Sens. Actuators, B*, 1990, **1**, 272.
- 25 E. J. R. Sudhölter, P. D. van der Wal, M. Skowronska-Ptasinka, A. van den Berg, P. Bergveld and D. N. Reinhoudt, *Anal. Chim. Acta*, 1990, **230**, 59.
- 26 J. G. Schindler and M. M. Schindler, *Fresenius Z. Anal. Chem.*, 1989, **335**, 553.
- 27 J. W. Ross, *U.S. Pat.* 80-211351, Nov. 28, 1980, GO1N027-30.
- 28 K. Tóth, E. Lindner, E. Pungor, E. Zippel and R. Kellner, *Fresenius Z. Anal. Chem.*, 1988, **331**, 448.
- 29 O. Dinten, U. E. Spichiger, N. Chaniotakis, P. Gehrig, B. Rusterholtz, W. E. Morf and W. Simon, *Anal. Chem.*, 1991, **63**, 596.

Paper 2/04119G; Received 30th July, 1992